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By: Jo Ann Yamila Dallara

PATENT
Attorney Docket No.: 019957-013211US
Client Ref. No.: 100/162

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: GILBERT and WAKARCHUK Application No.: 10/058,636 Filed: January 29, 2002 For: LIPOPOLYSACCHARIDE α-2,3 SIALYLTRANSFERASE OF *CAMPYLOBACTER JEJUNI* AND ITS USES	Examiner: Not yet assigned Art Unit: 1645 COMMUNICATION UNDER 37 C.F.R. §§ 1.821-1.825 AND PRELIMINARY AMENDMENT

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Sir:

In response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, 37 C.F.R. §§ 1.821-1.825, mailed April 19, 2002, Applicants submit that the computer-readable form in the instant application is identical with the Sequence Listing filed in Application No. 09/272,960, filed July 9,1999. In accordance with 37 C.F.R. § 1.821(e), please use the computer-readable form filed in Application No. 09/272,960 as the computer-readable form for the instant application. The information in the paper copy of

the Sequence Listing enclosed herewith is identical to that which is in the computer readable form, as required under 37 C.F.R. § 1.821(f).

It is understood that the Patent and Trademark Office will make the necessary changes in application number and filing date for the computer-readable form that will be used for the instant application.

Please amend the specification in adherence with 37 C.F.R. §§ 1.821-1.825 as follows.

In the Specification:

Please replace the paragraph beginning at page 4, line 2, with the following:

--The invention provides nucleic acid molecules that include a polynucleotide sequence that encodes an α2,3-sialyltransferase polypeptide. The α2,3-sialyltransferase polypeptide has an amino acid sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:2 over a region at least about 50 amino acids in length when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The polynucleotide sequences are preferably at least about 75% identical to a polynucleotide sequence of a *Campylobacter jejuni* α2,3-sialyltransferase gene as set forth in SEQ ID NO:1 over a region at least about 120 nucleotides in length when compared using the BLASTN algorithm with a wordlength (W) of 11, M=5, and N= -4. The nucleic acid molecules of the invention will generally hybridize to a polynucleotide sequence of SEQ ID NO:1 under stringent conditions.--

Please replace the paragraph beginning at page 4, line 13, with the following:

--The invention also provides isolated α2,3-sialyltransferase polypeptides that have an amino acid sequence at least about 75% identical to the amino acid sequence

of a *Campylobacter jejuni* α2,3-sialyltransferase as set forth in SEQ ID NO:2, over a region at least about 50 amino acids in length, when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The invention provides, in one embodiment, full-length sialyltransferase polypeptides that have about 430 amino acids. Also provided are truncated sialyltransferase polypeptides that are at least about 328 amino acids in length and also have sialyltransferase activity.--

Please replace the paragraph beginning at page 5, line 14, with the following:

--**Error! Reference source not found.** shows an alignment of the deduced amino acid sequences of the *C. jejuni* OH4384 c*st-I* gene (CST-I; SEQ ID NO:2) and an *H. influenzae* putative ORF (HIN; SEQ ID NO:5) (GenBank #U32720). The alignment was performed using the ALIGN program (Genetics Computer Group, Madison WI). The solid vertical lines between the sequences show identical residues.--

Please replace the paragraph beginning at page 12, line 15, with the following:

--The invention provides nucleic acid molecules that include a polynucleotide sequence that encodes an α2,3-sialyltransferase polypeptide that have an amino acid sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:2. The region of identity is typically over a region at least about 50 amino acids in length when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The region of identity extends more preferably over at least about 200 amino acids, still more preferably over at least about 328 amino acids, and most preferably over the full length of the polypeptide.--

Please replace the paragraph beginning at page 12, line 23, with the following:

--The polynucleotide sequences are typically at least about 75% identical to a polynucleotide sequence of a *Campylobacter jejuni* α2,3-sialyltransferase gene such as that set forth in SEQ ID NO:1. The region of similarity between the nucleic acid molecules of the invention and the *C. jejuni* sialyltransferase sequence extends over at least about 120 nucleotides, preferably over at least about 500 nucleotides, and most preferably extends over the entire length of the sialyltransferase coding region. To identify nucleic acids of the invention, one can employ a nucleotide sequence comparison algorithm such as are known to those of skill in the art. For example, one can use the BLASTN algorithm. Suitable parameters for use in BLASTN are a wordlength (W) of 11, M=5, and N= -4. Alternatively, one can identify a nucleic acid of the invention by hybridizing, under stringent conditions, the nucleic acid of interest to a nucleic acid that includes a polynucleotide sequence of SEQ ID NO:1. One example of a nucleic acid of the invention includes a polynucleotide sequence of a *C. jejuni* α2,3-sialyltransferase enzyme as set forth in SEQ ID NO:1.--

Please replace the paragraph beginning at page 14, line 10, with the following:

--The desired nucleic acids can also be cloned using well known amplification techniques. Examples of protocols sufficient to direct persons of skill through *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.* (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3: 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86: 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87: 1874; Lomell *et al.* (1989) *J. Clin. Chem.* 35: 1826; Landegren

et al. (1988) *Science* 241: 1077-1080; Van Brunt (1990) *Biotechnology* 8: 291-294; Wu and Wallace (1989) *Gene* 4: 560; and Barringer *et al.* (1990) *Gene* 89: 117. Improved methods of cloning *in vitro* amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039. Suitable primers for use in the amplification of the nucleic acids of the invention include, for example:

CJ18F: 5' primer of *C. jejuni* α-2,3-STase (SEQ ID NO:3) (41 mer, NdeI site in italics)

5' C TTA GGA GGT *CAT ATG* ACA AGG ACT AGA ATG GAA AAT GAA C 3'

CJ40R: 3' primer of *C. jejuni* α-2,3-STase (SEQ ID NO:4) with 6 His tail (60 mer, *SalI* site in italics, $(His)_6$ (SEQ ID NO:6) tag in bold)

5' CC TAG *GTC GAC* TCA TTA **GTG GTG ATG GTG GTG ATG** TTC CCC TTT CTC AAA CTC TCT CTT C 3'.--

Please replace the paragraph beginning at page 15, line 19, with the following:

--The invention also provides α2,3-sialyltransferase enzymes. The α2,3-sialyltransferase polypeptides of the invention typically have an amino acid sequence that is at least about 75% identical to an amino acid sequence of a *C. jejuni* α2,3-sialyltransferase as set forth in SEQ ID NO:2. The region of similarity between a *C. jejuni* sialyltransferase and a polypeptide of interest typically extends over a region at least about 50 amino acids in length, more preferably over at least about 200 amino acids, still more preferably over at least about 328 amino acids, and most preferably over the full length of the polypeptide. One example of an algorithm that is useful for comparing a polypeptide to the amino acid sequence of a *C. jejuni* α2,3-sialyltransferase is the BLASTP algorithm; suitable parameters include a wordlength (W) of 3, and the BLOSUM62 scoring matrix. One example of a sialyltransferase polypeptide of the invention has an amino acid sequence as set forth in SEQ ID NO:2.--

Please replace the paragraph beginning at page 25, line 13, with the following:

--The *malE* gene (GenBank #AE000476) without its signal peptide was obtained by PCR amplification from *E. coli* BMH genomic DNA using primers that added a *Bam*HI restriction site on the 5' end and an *Nde*I site on the 3' end. These two restriction sites allowed the gene to be inserted in the expression vector pCW (Wakarchuk *et al.* (1994) *Protein Sci.* 3: 467-475) immediately in front of the *cst-I* gene with a Gly-Gly-Gly-His (SEQ ID NO:7) linker between the two domains. The fusion proteins were purified on commercially available amylose resin (New England Biolabs) using a protocol suggested by the manufacturer. Maltose was removed by dialysis of the eluted protein against 50 mM HEPES-NaOH pH 7.5.--

Please cancel the "SEQUENCE LISTING", pages 1-3, submitted on July 9, 1999, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 6, at the end of the application. Please renumber the pages for the Claims and Abstract, accordingly.

REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. The information contained in the computer readable form of Application No. 09/272,960, filed July 9,1999, was prepared through the use of the software program "PatentIn" and was identical to that of the paper copy which was printed from the floppy disk, a copy of which is enclosed for the convenience of the Examiner. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification and Claims by the current Amendment. The attached pages are captioned **"VERSION WITH MARKINGS TO SHOW CHANGES MADE."**

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Kevin L. Bastian
Reg. No. 34,774

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 2 of page 4 has been amended as follows:

The invention provides nucleic acid molecules that include a polynucleotide sequence that encodes an α2,3-sialyltransferase polypeptide. The α2,3-sialyltransferase polypeptide has an amino acid sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:2 ~~SEQ. ID. NO: 2~~ over a region at least about 50 amino acids in length when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The polynucleotide sequences are preferably at least about 75% identical to a polynucleotide sequence of a *Campylobacter jejuni* α2,3-sialyltransferase gene as set forth in SEQ ID NO:1 ~~SEQ. ID. NO:1~~ over a region at least about 120 nucleotides in length when compared using the BLASTN algorithm with a wordlength (W) of 11, M=5, and N= -4. The nucleic acid molecules of the invention will generally hybridize to a polynucleotide sequence of SEQ ID NO:1 ~~SEQ. ID. NO: 1~~ under stringent conditions.

Paragraph beginning at line 13 of page 4 has been amended as follows:

The invention also provides isolated α2,3-sialyltransferase polypeptides that have an amino acid sequence at least about 75% identical to the amino acid sequence of a *Campylobacter jejuni* α2,3-sialyltransferase as set forth in SEQ ID NO:2 ~~SEQ. ID. No. 2~~, over a region at least about 50 amino acids in length, when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The invention provides, in one embodiment, full-length sialyltransferase polypeptides that have about 430 amino acids. Also provided are truncated sialyltransferase

polypeptides that are at least about 328 amino acids in length and also have sialyltransferase activity.

Paragraph beginning at line 14 of page 5 has been amended as follows:

Error! Reference source not found. shows an alignment of the deduced amino acid sequences of the *C. jejuni* OH4384 c*st-I* gene (CST-I; SEQ ID NO:2) and an *H. influenzae* putative ORF (HIN; SEQ ID NO:5) (GenBank #U32720). The alignment was performed using the ALIGN program (Genetics Computer Group, Madison WI). The solid vertical lines between the sequences show identical residues.

Paragraph beginning at line 15 of page 12 has been amended as follows:

The invention provides nucleic acid molecules that include a polynucleotide sequence that encodes an α2,3-sialyltransferase polypeptide that have an amino acid sequence that is at least about 75% identical to an amino acid sequence as set forth in SEQ ID NO:2 ~~SEQ. ID. NO: 2~~. The region of identity is typically over a region at least about 50 amino acids in length when compared using the BLASTP algorithm with a wordlength (W) of 3, and the BLOSUM62 scoring matrix. The region of identity extends more preferably over at least about 200 amino acids, still more preferably over at least about 328 amino acids, and most preferably over the full length of the polypeptide.

Paragraph beginning at line 23 of page 12 has been amended as follows:

The polynucleotide sequences are typically at least about 75% identical to a polynucleotide sequence of a *Campylobacter jejuni* α2,3-sialyltransferase gene such as that set forth in SEQ ID NO:1 ~~SEQ. ID. NO:1~~. The region of similarity between the nucleic acid molecules of the invention and the *C. jejuni* sialyltransferase sequence extends over at least about 120 nucleotides, preferably over at least about 500

nucleotides, and most preferably extends over the entire length of the sialyltransferase coding region. To identify nucleic acids of the invention, one can employ a nucleotide sequence comparison algorithm such as are known to those of skill in the art. For example, one can use the BLASTN algorithm. Suitable parameters for use in BLASTN are a wordlength (W) of 11, M=5, and N= -4. Alternatively, one can identify a nucleic acid of the invention by hybridizing, under stringent conditions, the nucleic acid of interest to a nucleic acid that includes a polynucleotide sequence of SEQ ID NO:1 ~~SEQ. ID. NO: 1~~. One example of a nucleic acid of the invention includes a polynucleotide sequence of a *C. jejuni* α2,3-sialyltransferase enzyme as set forth in SEQ ID NO:1.

Paragraph beginning at line 10 of page 14 has been amended as follows:

The desired nucleic acids can also be cloned using well known amplification techniques. Examples of protocols sufficient to direct persons of skill through *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.* (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3: 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86: 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87: 1874; Lomell *et al.* (1989) *J. Clin. Chem.* 35: 1826; Landegren *et al.* (1988) *Science* 241: 1077-1080; Van Brunt (1990) *Biotechnology* 8: 291-294; Wu and Wallace (1989) *Gene* 4: 560; and Barringer *et al.* (1990) *Gene* 89: 117. Improved methods of cloning *in vitro* amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039. Suitable primers for use in the amplification of the nucleic acids of the invention include, for example:

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CJ40R: 3' primer of *C. jejuni* α-2,3-STase (SEQ ID NO:4) with 6 His tail (60 mer, *SalI* site in italics, $(His)_6$ (SEQ ID NO:6) tag in bold) ~~(SEQ ID NO:4)~~

5' CC TAG *GTC GAC* TCA TTA **GTG GTG ATG GTG GTG ATG** TTC CCC TTT CTC AAA CTC TCT CTT C 3'

Paragraph beginning at line 19 of page 15 has been amended as follows:

The invention also provides α2,3-sialyltransferase enzymes. The α2,3-sialyltransferase polypeptides of the invention typically have an amino acid sequence that is at least about 75% identical to an amino acid sequence of a *C. jejuni* α2,3-sialyltransferase as set forth in SEQ ID NO:2 ~~SEQ. ID. NO: 2~~. The region of similarity between a *C. jejuni* sialyltransferase and a polypeptide of interest typically extends over a region at least about 50 amino acids in length, more preferably over at least about 200 amino acids, still more preferably over at least about 328 amino acids, and most preferably over the full length of the polypeptide. One example of an algorithm that is useful for comparing a polypeptide to the amino acid sequence of a *C. jejuni* α2,3-sialyltransferase is the BLASTP algorithm; suitable parameters include a wordlength (W) of 3, and the BLOSUM62 scoring matrix. One example of a sialyltransferase polypeptide of the invention has an amino acid sequence as set forth in SEQ ID NO:2.

Paragraph beginning at line 13 of page 25 has been amended as follows:

The *malE* gene (GenBank #AE000476) without its signal peptide was obtained by PCR amplification from *E. coli* BMH genomic DNA using primers that added a *Bam*HI restriction site on the 5' end and an *Nde*I site on the 3' end. These two restriction sites allowed the gene to be inserted in the expression vector pCW (Wakarchuk *et al.* (1994) *Protein Sci.* **3**: 467-475) immediately in front of the *cst-I* gene with a Gly-Gly-Gly-His (SEQ ID NO:7) linker between the two domains. The fusion proteins were purified on commercially available amylose resin (New England Biolabs) using a protocol suggested by the manufacturer. Maltose was removed by dialysis of the eluted protein against 50 mM HEPES-NaOH pH 7.5.